

# **Division of Intramural Research**

## **NAEHS Council Update**

**September 2003**

## **DIR RECRUITMENTS**

### **Senior Molecular Toxicologist**

The Environmental Toxicology Program is conducting a search for a senior tenured investigator to direct research in molecular toxicology. The candidate will be expected to develop and maintain a strong intramural research effort in toxicology, particularly as it relates to defining critical target pathways, genes and cellular/molecular mechanisms of target organ responses to environmental factors and to provide programmatic leadership and council to the initiatives of the Environmental Toxicology and the National Toxicology Program in the candidate's area of expertise. Researchers in the area of developmental toxicology are particularly sought, although qualified individuals in any area of toxicological research are encouraged to apply. The Candidate should be a senior investigator with an international reputation for cutting edge research within the broad context of toxicology, an outstanding publications record, a proven history of research leadership, and demonstration of knowledge of toxicology and human health issues. Dr. Robert Maronpot, Chief of the Laboratory of Experimental Pathology, is the Chair of the search committee.

### **Tenure-track Bioinformaticist**

The Biostatistics Branch is conducting a nationwide search for a tenure-track investigator with training and experience in bioinformatics. The person selected will focus activities upon developing novel methods related to toxicogenomics, such as developing and evaluating data mining approaches for elucidating characteristic patterns in gene expression array or proteomic data in order to facilitate searches for functionally-coordinated families of genes related to disease processes or response to toxicants. Improved quantitative methods for functional genomics and data mining are needed to make full scientific use of the toxicogenomics data being produced by the NIEHS Microarray Center and the National Center for Toxicogenomics. A search committee chaired by Dr. Douglas Bell, Laboratory of Computational Biology and Risk Analysis has recommended candidates to the Scientific Director.

### **Tenure-track Immunologist**

The Laboratory of Pulmonary Pathobiology is conducting a national search for a cellular/molecular immunologist. The candidate will be expected to establish a high-quality independent research program in pulmonary immunology in a laboratory with diverse research interests and backgrounds. The successful candidate will have research strengths in, but not necessarily limited to, pulmonary biology (such as mechanisms of tolerance, allergy, adaptive and/or innate immune response to respiratory infections, etc). A search committee chaired by Dr. John Drake, Chief of the Laboratory of Molecular Genetics has recommended a candidate and negotiations are underway.

### **Tenure-track Environmental Epidemiologist**

The Epidemiology Branch has conducting a national search for an environmental epidemiologist. This person will be expected to develop an outstanding research program on the effects of environmental exposures and risks of chronic disease. Applicants with

demonstrated research interests in biological mechanisms and etiology of (not limited to) neurodegenerative diseases, diabetes, multiple sclerosis, renal disease, cardio-respiratory diseases; and such exposures as pesticides, metals, and/or solvents are most welcome. A search committee chaired by Dr. Steven Kleeberger, Chief of the Laboratory of Pulmonary Pathobiology is interviewing candidates.

**Tenure-track or Tenured Biostatistician--Statistical Genetics**

The Biostatistics Branch has conducted an international search for a tenure-track or tenured statistician to conduct independent research on methods development in statistical genetics. The successful candidate will be expected to develop statistical methods for family-based studies aimed at identifying and mapping genes that influence risk modifying quantitative traits or diseases or that interact with the environmental agents that cause human disease. An offer has been extended to a leading candidate.

**Staff Scientist--Toxicologic Pathologist**

The Laboratory of Experimental Pathology is conducting a national search for a toxicologic pathologist to provide support and peer review for the National Toxicology Program toxicity and carcinogenicity studies and to provide support for NIEHS researchers. A search committee chaired by Dr. Rick Hailey, Toxicology Operations Branch, is interviewing candidates.

**Staff Scientist—Pathologist/Laboratory Animal Veterinarian**

The Laboratory of Experimental Pathology is conducting a national search for a laboratory animal veterinarian to provide management, oversight, production support, genetic monitoring and disease surveillance of laboratory animals for the National Toxicology Program. A search committee chaired by Dr. Joseph Roycroft, Toxicology Operations Branch, is interviewing candidates.

**Staff Scientist—Toxicogenomics**

The National Center for Toxicogenomics (NCT) of the National Institute of Environmental Health Sciences is conducting a national search for a Staff Scientist to lead a core facility to support a research program to direct the basic research applications of gene expression technologies within the NCT. The NCT is conducting an aggressive research program to apply genomic technology to toxicology and to facilitate the identification of biomarkers of specific adverse effects of exposure to environmental agents including drugs, chemicals, and stressors. The activities of the Center will enable other investigators to probe the complexities of the mechanisms of normal genetic and metabolic pathways and to subsequently learn how diseases occur when these pathways malfunction. The position will be filled at the level of a Staff Scientist who will work in support of existing research programs in the Institute's Division of Intramural Research. A search committee is being formed.

**Staff Scientist—Bioethics**

The Office of Clinical Research is conducting a national search for a bioethicist to be involved with health policy research on the effectiveness of federal and Institutional Review Board regulations in addressing clinical studies and clinical genetics issues. A

search committee chaired by Dr. Stephanie London, Epidemiology Branch, has been formed and the position has been advertised.

## DIR RECRUITS

### **Dr. Grace Kissling**

#### **Staff Scientist—Biostatistics Branch**

Dr. Kissling has recently joined the NIEHS from a faculty position at the University of North Carolina-Greensboro. She will be analyzing data from NTP studies, providing statistical consulting services to researchers across the institute, and developing new statistical methodology where needed. Dr. Kissling has experience collaborating as a statistical consultant in research projects from a wide range of disciplines, including anthropology, biology, cardiology, education research, history, internal medicine, pharmacy, physical therapy, psychology, music, nursing, nutrition, sport science, and textiles.

Dr. Kissling's statistical research involves developing methods for assessing spatial association of measurements whose geographic locations are known. Several spatial autocorrelation measures have been proposed in the past. She has extended one of these measures, Moran's I statistic, in two ways--first, she developed a spatial autocorrelation coefficient for multivariate data so that a spatial "autocovariance" might shed light on how two or more continuous variables co-vary over space; second, for categorical measurements, she developed a measure of the degree of agreement of categories, taking into account the spatial proximity of the observations.

#### *Selected publications*

- Brodie, B.R., T. D. Stuckey, C. Hansen, G. Kissling, and D. Muncy. Influence of vessel size on early and late outcomes after primary angioplasty for acute myocardial infarction, *J. Invas. Cardiol.* 12:13-19, (2000).
- C. G. Gegick, M. D. Altheimer, G. E. and Kissling. "Benefits of outcome analysis in diabetes management, *Endocrin. Pract.*, 6:253-259 (2000).
- S. A. Beeson and G. E. Kissling. Predicting success for baccalaureate graduates on the NCLEX-RN#2, *J. Prof. Nurs.*, 17: 121-127, (2001).
- C. K. Miller, L. Edwards, G. Kissling, and L. Sanville. Evaluation of a theory-based nutrition intervention for older adults with diabetes mellitus, *J. Amer. Diet. Assoc.* 102:1069-1081, (2002).
- M. K. Sandford, G. Bogdan, and G. E. Kissling. Biological adaptation in the prehistoric Caribbean: Osteology and bioarchaeology of the Tutu site, in E. Righter (ed.) *Human Adaptations at the Tutu Archaeological Village Site: A Multidisciplinary Case Study*, Gordon and Breach Science Publishers, New York, 209-229 (2002).

## **DIR SCIENTIFIC ACCOMPLISHMENTS 2003**

### **DDT Might Increase Infant Mortality When Used To Control Malaria**

Sub-Saharan African countries have sought exemptions from the worldwide ban on DDT to spray houses for malaria control. DDT is not acutely toxic to humans, but data from NIEHS epidemiology studies have shown that DDT may increase low birthweight and interfere with prolonged breastfeeding, both of which would increase infant mortality. NIEHS epidemiologists have shown that the cost in increased infant mortality from DDT toxicity was of the same order of magnitude as the benefit from infant malaria deaths prevented, if the associations are causal and of the same strength in Africa as seen in North America. Thus programs that propose to use DDT should have sufficient research capability to see if they are having the hoped-for effect, and alternative treatments, such as insecticide treated bed nets, should be reconsidered in the light of these findings.

Chen, A. and Rogan, W.J.: Nonmalarial Infant Deaths and DDT Use for Malaria Control. *Emerg. Infect. Dis.* 9: in press, 2003.

### **A New Way To Cause Mutations and Cancer by Environmental Factors.**

Cadmium, which is a known human carcinogen, was found to cause extreme hypermutability in yeast following chronic exposure to low amounts that are similar to those that accumulate in the human body and can be found in the environment. Genetic analysis in yeast and direct biochemical testing in human cell extracts demonstrated that cadmium is a new kind of mutagen that acts by inhibiting the DNA mismatch repair system rather than via direct DNA damage. It was believed for many years that environmental mutagens may have their effects not only through directly damaging DNA, but also by altering the physiology of genome stability maintenance, but there has never been a clear identification of the biological targets. Environmental alteration of key DNA damage response pathways may prove as important as direct DNA damage by mutagens.

Jin Y.H., Clark A.B., Slebos R.J., Al-Refai H., Taylor J.A., Kunkel T.A., Resnick M.A. and Gordenin D.A.: Cadmium is a mutagen that acts by inhibiting mismatch repair. *Nature Genet.*, 34: 326-329, 2003.

### **Grandparents Have a Lot to Tell Us About Genetic Contributions to Early-onset Disease.**

Diseases with onset early in life, such as birth defects, insulin-dependent diabetes, and schizophrenia, are known to depend on both genetic and environmental factors. The genetic variations that confer increased susceptibility to such "complex" diseases have been difficult

to identify. NIEHS statisticians developed a method to locate risk-related genes by studying the transmission of genes from grandparents through the parents to affected grandchildren. Each copy of a gene that we carry represents a random choice from

among the 8 copies that were present in our 4 grandparents. However, if a variant form of the gene is related to increased risk of the disease under study, then that variant will have been inherited by diseased grandchildren with a likelihood higher than chance. We developed powerful statistical methods to identify such apparent transmission distortions and estimate the relative risks associated with the variant alleles.

Weinberg, C.R.: Studying parents and grandparents to assess genetic contributions to early-onset disease. *Am. J. Hum. Gen.* 72: 438-47, 2003.

### **The Insecticide DDT is a Reproductive Toxin In Humans.**

DDT is still used for control of malaria in dozens of countries worldwide. Ongoing use of DDT is endorsed by the World Health Organization, based on the assumption that it has little or adverse effects on humans. New evidence, however, suggests that like birds and other animals, humans experience reproductive toxicity when exposed to the levels of DDT encountered in insect control programs. International policy regarding this persistent organic pollutant needs to be reconsidered.

Longnecker, M.P., Klebanoff, M.A., Dunson, D.B., Guo, X., Chen, Z., Zhou, H. and Brock, J.W.: Maternal serum level of the DDT metabolite DDE in relation to fetal loss in previous pregnancies. *Environ. Res.*, in press, 2003.

### **Elimination of Mercury and Methyl-mercury Accelerated by Kidney Transport Proteins.**

Our bodies are protected from the toxic effects of mercury and methyl-mercury by their rapid reaction with endogenous sulfur-containing molecules or by antidotes including dimercapto-propane-sulfonate (DMPS). An important component of these protective effects was shown by NIEHS pharmacologists to be the rapid excretion of mercury-sulfhydryl compounds mediated by the kidney drug and xenobiotic transport protein known as organic anion transporter 1 (OAT1). Thanks to the action of this excretory protein the body burden of mercury, and thus its toxicity, is greatly reduced.

Koh, A.S., Simmons-Willis, T.A., Pritchard, J.B., Grassl, S.L. and Ballatori, N.: Identification of a mechanism by which the methylmercury antidotes N-acetylcysteine and dimercapto-propane-sulfonate enhance urinary metal excretion: Transport by the renal organic anion transporter-1, Oat1, but not by Oat3. *Molec. Pharmacol.* 62: 921-926, 2002.

Aslamkhan, A.G., Han, Y-H., Yang, X-P, Zalups, R.K. and Pritchard, J.B.: Human renal organic anion transporter 1 (hOAT1) dependent uptake and toxicity of mercuric thiol-conjugates in MDCK cells. *Molec. Pharmacol.* 63: 590-596, 2003.

**New Genetic Defects Identified in Human Drug Metabolizing Enzymes That Potentially Alter Dosage Requirements, Cure Rates and Toxicity for Prescription Drugs and Over-the-Counter Remedies.**

NIEHS scientists have been identified genetic defects in two enzymes, CYP2C9 and CYP2C19, which metabolize approximately 16% of clinically used drugs including anticoagulents, anticonvulsant drugs used for epilepsy, antidiabetic drugs, drugs for high blood pressure, anti-inflammatory drugs such as celebrex and ibuprofen, the popular antiulcer agent omeprazole, and valium. These defects potentially cause variability in effective dosages, cure rates for ulcers, and drug toxicity in different individuals. Discovery of the majority of defective alleles opens the possibility of using genetic testing in the future to customize drug prescription to the patient to avoid toxicity and assure that drugs will be effective in the particular patient.

- Blaisdell, J., Mohrenweiser, H., Jackson, J., Ferguson, S., Coulter, S., Chanas, B., Xi, Ti, Ghanayem, B. and Goldstein, J.A.: Identification and functional characterization of new potentially defective alleles of human CYP2C19. *Pharmacogenetics* 12: 703-711, 2002.
- Goldstein, J.A.: Polymorphisms in the Human CYP2C Subfamily. *Drug Metabolism Reviews* 34: 5, 2002.
- Lee, C.R., Goldstein, J.A. and Pieper, J.A.: Cytochrome P450 2C9 Genetic Polymorphisms: a Comprehensive Review of the In Vitro and Human data. *Pharmacogenetics*, 12: 251-263, 2002.
- Lee, C.R., Pieper, J.A., Frye, R.F., Hinderliter, A.L., Blaisdell, J.A. and Goldstein, J.A.: Tolbutamide, Flurbiprofen and Losartan as probes of CYP2C9 activity in humans. *J. Clin Pharm.*, 43: 84-91, 2003.
- Fischer, T.L., Pieper, J.A., Graff, D.W., Rodgers, J.E., Fischer, J.D., Parnell, K.J., Goldstein, J.A., Greenwood, R. and Patterson, J.H.: Evaluation of potential losartin-phenytoin drug interactions in healthy volunteers. *Clin Pharm. Ther.* 72: 238-46, 2002.

**Dust Mite Allergen Exposure is Common In U.S. Homes.**

Dust mite allergen is perhaps one of the most studied indoor allergens but levels of exposures to dust mite allergen in U.S. homes had not been previously described. NIEHS scientists reported that 84% US homes have detectable levels of dust mite allergen in at least one bed. Levels previously associated with allergic sensitization and asthma morbidity are common in U.S. bedrooms. Independent predictors of higher dust mite allergen concentrations include older homes, regions other than the West, single-family homes, the absence of resident children, lower household income, a musty or mildew odor in the home, and higher bedroom humidity.

- Arbes, S.J., Friedman, W., Vojta, P.J., Muilenberg, M.L., Burge, H.A., Yin, M., Cohn, R. and Zeldin, D.C.: House dust mite allergens in U.S. beds: results from the first national survey of lead and allergens in housing. *J. Allergy Clin. Immunol.* 111: 408-414, 2003.

**Breastfeeding Protects Young Children Against Asthma and Recurrent Wheezing.**

Using data from the third National Health and Nutrition Examination Survey, NIEHS researchers found evidence that breastfeeding may delay the onset of, or actively protect children less than 24 months of age against, asthma and recurrent wheeze. Compared to children who had never breastfed, breastfed children had significantly reduced chance of being diagnosed with asthma and of having recurrent wheeze before 24 months of age. Also, among children 2 to 71 months of age who had been exposed to environmental tobacco smoke, those who had ever been breastfed had significantly reduced risks of asthma and wheeze compared with those who had never been breastfed.

Chulada, P.C., Arbes, S.J., Dunson, D. and Zeldin, D.C.: Breastfeeding and the prevalence of asthma and wheeze in children: analysis from the third national health and nutrition examination survey (NHANES III), 1988-1994. *J. Allergy Clinl. Immunol.* 111: 328-336, 2003.

**Hydrocephalus Gene Discovery.**

NIEHS researchers identified a novel gene called RFX4 that is critical for normal brain development. Loss of a single copy of this gene in mice results in severe congenital hydrocephalus (brain swelling) due to stenosis of the aqueduct of Sylvius and failure of formation of the subcommissural organ. Loss of both copies of this gene results in a severe brain defects and death in the perinatal period. These studies raise the possibility that similar defects in the expression of this gene in man might lead to congenital hydrocephalus, a disease that is present in 0.5-1.8 per 1000 live births.

Blackshear, P.J., Graves, J., Stumpo, D.J., Cobos, I., Rubenstein, J.L., and Zeldin, D.C. Graded phenotypic response to partial and complete deficiency of a brain-specific isoform of the winged helix transcription factor RFX4. *Development.* In Press, 2003.

**Mechanism of Glucocorticoid Resistance Described.**

Glucocorticoids are a class of drugs used for treatment of many inflammatory and autoimmune diseases caused by environmental stimuli. Glucocorticoid resistance is a pathological state characterized by the inability of glucocorticoids to elicit a normal pharmacological or physiological response and is associated with poor therapeutic outcome. Overexpression of the beta isoform of the human glucocorticoid receptor is associated with glucocorticoid resistance. NIEHS scientists described a molecular mechanism that can explain the properties of glucocorticoid receptor beta and its potential role in glucocorticoid resistance. Using atomic scale, three-dimensional modeling and molecular biology techniques, they have characterized how two specific amino acids in glucocorticoid receptor beta are responsible for its dominant negative phenotype and its suspected role in glucocorticoid resistance. This work may lead to novel therapeutic targets of glucocorticoid receptor beta that may result in new therapies for overcoming glucocorticoid resistance.

Yudt, M.R., Jewell, C.M., Bienstock, R.J. and Cidlowski, J.A.: Molecular origins for the dominant negative function of human glucocorticoid receptor beta. *Mol. Cell. Biol.* 23:4319-4330, 2003.

**New Method Reduces Exposure to Cockroach Allergen in Inner-City Homes.**

Clinically relevant reductions in exposure to cockroach allergen, an important risk factor for asthma in inner-city households, have proven very difficult to achieve in intervention trials. Using a randomized study NIEHS researchers investigated new methods for the abatement of cockroach allergen in low-income, urban homes. Interventions consisted of occupant education, placement of insecticide bait, and professional cleaning. Substantial reductions in cockroach allergen levels were achieved. Allergen levels were reduced below the sensitization threshold in beds, arguably the most relevant site for exposure, and below the asthma morbidity threshold on bedroom floors and living room floors/sofas.

Arbes, S.J., Sever, M., Archer, J., Long, E.H., Gore, C., Schal, C., Walter, M., Neubler, B., Vaughn, B., Mitchell, H. Liu, E., Collette, N., Adler, P. and Zeldin, D.C.: Abatement of cockroach allergen (blag1) in low-income, urban housing – A randomized control trial. *J. Allergy Clin. Immunol.* In press, 2003.

**Power Frequency Magnetic Fields Have No Effect on Breast Cancer Incidence**

Some previously published data suggest that residential exposure to power frequency magnetic fields may increase risk of breast cancer. NIEHS epidemiologists have found in a subset of the Hawaii and Los Angeles Multiethnic Cohort that higher residential exposure to magnetic fields did not increase breast cancer risk.

London, S.J., Pogoda, J.M., Hwang, K.L., Langholz, B., Monroe, K.R., Kolonel, L.N., Kaune, W.T., Peters, J.M. and Henderson, B.: Residential magnetic field exposure and breast cancer risk in the Multiethnic Cohort Study. *Amer. J. Epidemiol.*, in press, 2003.

**Mice and Men: Gene Mutations Causing Male Infertility.**

Men with a syndrome called dysplasia of the fibrous sheath (DSF) are infertile and have sperm with a short, rigid, and immotile tail. DSF occurs in several men in some families, evidence that it is caused by a genetic mutation. The fibrous sheath forms a skeletal framework for the sperm tail and it is under-developed and disorganized in DSF sperm. AKAP4 is a major protein of the fibrous sheath in mice and men. NIEHS researchers have shown that, when AKAP4 was mutated experimentally in mice, their sperm were remarkably similar to sperm in DSF patients. This study suggests that men with DSF should be screened for mutations in the AKAP4 gene and is one of the first to identify a candidate gene for a specific cause of male infertility.

Miki, K., Willis, W.D., Brown, P.R., Goulding, E.H., Fulcher, K.D. and Eddy, E.M.: Targeted disruption of the AKAP4 gene causes defects in sperm flagellum and motility. *Devel. Biol.* 248: 331-342, 2002.

**New Mechanism Identified for a Critical Drug Transport Protein Found in Kidney and the Blood-Brain Barrier.**

Human organic anion transporter 3 (hOAT3) is highly expressed in the kidney and the blood brain barriers. In expression systems, it was shown to be capable of transporting small (<500 Daltons) negatively charged drugs, foreign chemicals, and their metabolites. The mechanism used by this transport protein was poorly understood and thus, its importance in vivo for elimination of toxins from the brain or the body was not established. NIEHS researchers showed that transport was coupled to metabolic energy (via organic anion/dicarboxylate exchange) leading to markedly accelerated elimination of negatively charged chemicals or metabolites. In doing so, it demonstrated the critical importance of hOAT3 in protecting our bodies as a whole, and our brains in particular, from chemical toxicity.

Sweet, D.H., Chan, L.M.S., Walden, R., Yang, X-P., Miller, D.S. and Pritchard, J.B.: Renal organic anion transporter 3 (OAT3 [SLC22a8]) is a dicarboxylate exchanger indirectly coupled to the sodium gradient. *Am. J. Physiol.* 284: F763-F769, 2003.

**Identification of a Marker for Progenitor Cell Population in Mouse Skin - Implications in Gene Therapy and Carcinogenesis Research.**

Although it is a widely held belief that adult skin stem and progenitor cells reside in the bulge region of the hair follicle, no reliable marker has been identified to date that allows for direct isolation of this cell population. NIEHS scientists have recently demonstrated that the cell surface marker CD34 identifies this specific population of cells in mouse skin, and allows for the enrichment of living bulge cells, providing a unique opportunity for to study the behavior of these cells under experimental conditions. CD34-expressing hair follicles cells have characteristics unique to stem and progenitor cells--they are quiescent and have a high proliferative capacity--characteristics that are necessary for potential gene therapy and tissue engineering applications, as well as for the study of carcinogen target cells in the skin. This work represents the first use of a hair follicle bulge-specific marker that allows for positive selection of potential epidermal progenitor cells.

Trempeus, C.S., Morris, R.J., Bortner, C.D., Cotsarelis, G., Faircloth, R.S., Reece, J.M. and Tennant, R.W.: Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. *J. Invest. Dermatol.* 120: 501-511, 2003.

### **Global Ultraviolet Light May Alter Autoimmune Muscle Disease**

NIEHS clinical researchers coordinated a study that produced the first global findings from a group of international experts organized to utilize the natural genetic and environmental variations around the world to begin to understand differences in the clinical expression of, and genetic and environmental risk factors for, the autoimmune muscle disease, myositis. Myositis occurs in two major forms, dermatomyositis and polymyositis. Of the geoclimatic variables studied, surface ultraviolet radiation intensity most strongly predicted the relative proportion of dermatomyositis, and was strongly related to the proportion of the dermatomyositis autoantibodies at 15 locations on four continents. The striking differences in the proportion of dermatomyositis and dermatomyositis-specific autoantibodies observed around the world do not appear to be the result of inherent global variations in known genetic risk factors. These data suggest that ultraviolet light exposure modulates the expression of an autoimmune disease in different populations around the world. These findings have important preventative implications, may affect studies of other immune-mediated diseases, and suggest new avenues of investigation for such disorders.

Okada, S., Weatherhead, E. Targoff, I.N., Wesley, R. and Miller, F.W. for The International Myositis Collaborative Study Group: Global surface ultraviolet radiation intensity may modulate the clinical and immunologic expression of autoimmune muscle disease. *Arthritis Rheum.*, in press, 2003.

### **Genetically Modified Mouse Models (GEMM) Shown to Have Great Potential as Screening Tools for Identifying Human Cancer Causing Chemicals.**

GEMM have important advantages over conventional rodent bioassay screening paradigms including shorter duration and reduced animal requirements. However, uncertainties exist surrounding their sensitivity and predictiveness. In an extensive review of nearly 100 chemicals now tested in the three most studied GEMM, NIEHS scientists have shown that they identified a high percentage of the human carcinogens tested (80-90%) while maintaining a low background of “false positives” (non-carcinogens incorrectly identified as carcinogens). However, a number of “false negatives” (human carcinogens incorrectly identified as non-carcinogens) were seen. This analysis should provide the basis for further refinement of this important research and screening tool.

Pritchard, J.B., French, J.F., Davis, B.J. and Haseman, J.K.: Transgenic mouse models: Their role in carcinogen identification. *Environ. Hlth. Perspectives* 111: 444-454, 2003.

### **Analysis of Mutations of the P53 Tumor Suppressor has Implications for Evolution of Extensive Signaling Networks in Cells.**

Alterations in the tumor suppressor and stress-responsive p53 gene are associated with many tumors. Since p53 regulates over 50 “downstream” genes, which are part of a vast network of cellular processes including programmed cell division and death, changes in

p53 can have dramatic effects on normal metabolism. NIEHS geneticists developed a unique system using yeast to assess the impact of human p53 mutations on regulation of many genes. Surprisingly, mutations with altered function can lead to a change in the spectrum and the level of genes activated by p53. This result suggests that there may be master genes of diversity such as p53 that provide rapid evolution of cellular regulatory networks, which helps explain how specific p53 mutations may be selected for tumor development.

Inga, A., Storici, F., Darden, T.A. and Resnick, M.A.: Differential transactivation by the p53 transcription factor is highly dependent on p53 level and promoter target sequence. *Mol. Cell. Biol.*, 22: 8612-86125, 2002.

Resnick, M.A. and Inga, A.: Functional mutations in the sequence-specific transcription factor p53 and implications for master genes of diversity. *Proc. Nat. Acad. Sci. USA* in press, 2003.

### **Ion fluxes are Required for Apoptosis.**

Cell shrinkage is a ubiquitous characteristic of apoptosis that discriminates it from other types of cell death such as necrosis. NIEHS scientists have shown for the first time that influx of sodium ions is required for cell shrinkage and a loss in intracellular potassium is actually the critical event that controls the progression of apoptosis, regardless of whether cells shrink or swell. This novel finding indicates that cell shrinkage can now be separated from other features of apoptosis. Such a basic science result can provide new avenues of therapy for many of the over 40 human apoptotic diseases.

Bortner, C.D. and Cidlowski, J.A.: Uncoupling cell shrinkage from apoptosis reveals that Na<sup>+</sup> influx is required for volume loss during programmed cell death. *J. Biol. Chem.*, in press, 2003.

### **Ion Channel Regulation by Signal Transduction Pathways**

NIEHS scientists have reported novel mechanisms for potassium channel regulation by signal transduction pathways. These mechanisms provide new targets for investigating the disruptive effects of environmental toxicants on cell function. In particular, potassium channel stimulation by thyroid hormone through the Rac GTPase provides a direct molecular mechanism to explain the human neurological deficits associated with disruption of thyroid hormone signaling during development by dietary iodine deficiency, organochlorine exposure, and inherited mutations in the thyroid hormone receptor. In addition the regulation of cardiac calcium channels by dihydropyridines, which are used to treat human cardiovascular disease was studied.

Storey, N., O'Bryan, J. and Armstrong, D.L.: Rac and Rho mediate opposing hormonal regulation of ether-a-go-go related potassium channels. *Curr. Biol.*, 12: 27-33, 2002.

Erxleben, C., Everhart, A., Romeo, C., Florance, H., Bauer, M.B., Alcorta, D., Rossie, S., Shipston, M.J. and Armstrong, D.L.: Interacting effects of N-

- terminal variation and stress-exon splicing on rSlo potassium channel modulation by calcium, phosphorylation and oxidation. *J. Biol. Chem.*, 277: 27045-27052, 2002.
- Tian, L., Coghill, L.S., MacDonald, H-F., Armstrong, D.L. and Shipston, M.J.: Leucine zipper domain targets PKA to mammalian BKCa channels. *J. Biol. Chem.*, 278: 8669-8677, 2003.
- Storey, N.M, Gómez-Angelats, M., Bortner, C., Armstrong, D.L. and Cidlowski, J.A.: Stimulation of Kv1.3 potassium channels by death receptors in Jurkat T lymphocytes. *J. Biol. Chem.*, in press, 2003
- Erxleben, C. Allegria-Gomez, T. Darden, T., Mori, Y., Birnbaumer, L. and Armstrong, D.L.: Modulation of cardiac CaV1.2 channels by dihydropyridine and phosphatase inhibitor requires Ser-1142 in the domain III pore loop. *Proc. Natl. Acad. Sci. USA*, 100: 2929-2934, 2003.

### **Long Sought Understanding of Drug Transporter Function Achieved.**

Organic anion transporter 1 (Oat1) was the first anionic drug transporter cloned, but its precise mechanism remained elusive. Using membranes isolated from both native rat kidneys and cell lines expressing human OAT1, NIEHS pharmacologists have shown that drug uptake was mediated by exchange for the Krebs cycle intermediate,  $\alpha$ -ketoglutarate. Moreover, this exchange was 1 for 1, meaning that each transporter cycle results in net entry of one positive charge and providing a direct means of tapping the energy stored in the inside negative membrane potential present in the renal excretory cells. In this manner, the cell's energy is harnessed to drive the rapid and effective elimination of toxic foreign chemicals and their metabolites.

- Aslamkhan, A.G., Han, Y-H., Walden, R., Sweet, D.H. and Pritchard, J.B.: Stoichiometry of renal organic anion/dicarboxylate exchange: Assessment in membrane vesicles from rat renal cortex isolated from rat kidney and human OAT1- expressing MDCK cells. *Am. J. Physiol.*, in press, 2003.

### **Possible Cause of Progressive External Ophthalmoplegia Identified.**

NIEHS scientists have discovered an active site point mutation in the gene for the mitochondrial DNA polymerase gamma that is associated with the human genetic disease Progressive External Ophthalmoplegia, which results from mitochondrial dysfunction. This mutated gene encodes a polymerase with reduced catalytic efficiency and reduced DNA replication fidelity, features that are implicated as causative for this disease.

- Ponamarev, P., Longley, M.J., Nguyen, D., Kunkel, T.A. and Copeland, W.C.: Active site mutation in DNA polymerase gamma associated with Progressive External Ophthalmoplegia causes error-prone DNA synthesis. *J. Biol. Chem.* 277: 15225-15228, 2002.

**Identification of Deleted In Split Hand/Split Foot 1 (Dss1) as a TPA-Responsive Gene Expressed in Keratinocyte Stem/Progenitor Cells and Possible Involvement in Early Skin Tumorigenesis.**

The keratinocyte stem cells (KSCs) have become a major target for cutaneous carcinogens and skin tumorigenesis might be initiated by cellular transformation of KSCs. NIEHS scientists are identifying the specific promotion-relevant effector genes that might lead to neoplastic transformation in skin following treatment with tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) using genetically initiated Tg.AC mouse keratinocyte stem/progenitor cells. Dss1 was one of novel TPA-responsive genes in mouse skin KSCs and its expression was also shown to be elevated in skin papillomas relative to normal skins, and further increased in squamous cell malignancies. Functional studies by constitutive expression of Dss1 in JB6 Cl 41-5a preneoplastic epidermal cells strongly increased focus-formation and proliferation of these cells and enhanced efficiency of neoplastic transformation of the cells. Dss1 represents an attractive candidate mediator of TPA-induced tumor promotion. These results are of broad interests in the skin cancer fields as well as other related stem/progenitor cell biomedical research areas.

Wei, S.-J., Trempus, C.S., Cannon, R.E. Bortner, C.D. and Tennant, R.W.: Identification of Dss1 as a 12-O-tetradecanoylphorbol-13-acetate-responsive gene expressed in keratinocyte progenitor cells, with possible involvement in early skin tumorigenesis. *J. Biol. Chem.* 278: 1758-1768, 2003.

**The Structural Mechanism of Anti-viral Induced Mitochondrial Toxicity.**

AIDS patients undergoing antiviral therapy with nucleoside analogs such as AZT, D4T, and ddC, can develop mitochondrial toxicity due to the inhibition of the mitochondrial DNA polymerase. NIEHS scientists have determined how the human mitochondrial DNA polymerase selects and incorporates these nucleoside analogs into mitochondrial DNA to cause inhibition of mitochondrial function. A single amino acid in the active site of the DNA polymerase gamma provides for most of the selection of ddC and D4T and possible carbovir. This comprehensive study of inhibition by antiviral nucleotide analogs should be useful to the rational design of more effective anti-viral inhibitors that do not cause such deleterious effects on mitochondrial DNA replication.

Lim, S.E., Ponamarev, M.V., Longley, M.J. and Copeland W.C.: Structural determinants in DNA polymerase  $\gamma$  that account for mitochondrial toxicity from antiviral nucleotide analogs *J. Mol. Biol.* 329: 45-57, 2003.

**Molecular Modeling of Human DNA Repair Protein Gives Insight into Three Human Diseases.**

Structural studies of human proteins often give insights into disease processes. However, it is sometimes difficult or impossible to obtain sufficient human protein in high enough purity to obtain crystals that yield atomic resolution information. Therefore, molecular modeling is an extremely useful complementary approach when direct structural

information is lacking. Scientists at the NIEHS have built a detailed molecular model of a human DNA repair protein, XPD using the crystal structure of UvrB, a homologous repair protein from bacteria. Mutations in the XPD gene can lead to one of three human diseases: xeroderma pigmentosum, trichothiodystrophy, and Cockayne's Syndrome. XPD as part of a nine protein complex functions in a variety of cellular functions including, DNA repair, transcription and cell cycle control. The validity of the model was tested in two ways. First, mutations associated with these human diseases were introduced in the bacterial UvrB protein and these mutant proteins were tested in a series of biochemical DNA repair assays. Second, specific mutations were introduced into XPD and its activity tested in DNA repair and transcriptional assays. Mutations in specific regions of the protein were found to affect repair producing xeroderma pigmentosum, while other sites in the protein affect transcription, producing either trichothiodystrophy, or Cockayne's Syndrome.

Bienstock, R.J., Skorvaga, M., Mandavilli, B.S. and Van Houten, B.: Structural and functional characterization of the human DNA repair helicase XPD by comparative molecular modeling and site-directed mutagenesis of the bacterial repair protein UvrB. *J. Biol. Chem.*, 278: 5309 – 5316, 2003.

Dubaele, S., Proietti de Santis, L., Bienstock, R.J., Keriell, A., Stefanini, M., Van Houten, B. and Egly, J.M.: Transcription activity of TFIIH derived from XPD patients discriminates between Xeroderma Pigmentosum and Trichothiodystrophy *Molecular Cell* 11: 1635-46, 2003.

### **Pharmacological Activation of an Oxygen Sensor in Cells Confers Protection by Upregulating Protective Genes.**

Recently NIEHS scientists have identified an oxygen dependent prolyl hydroxylase domain-containing enzyme as an oxygen-sensing pathway. Hydroxylation of a specific proline in the hypoxic-inducible-factor by this enzyme results in an increased synthesis of a number of genes that protected the cell from additional stress.

Wright, G., Higgins, J.J., Raines, R.T., Steenbergen, C. and Murphy, E.: Activation of the Prolyl Hydroxylase Oxygen-sensor results in induction of GLUT1, heme oxygenase-1, and nitric-oxide synthase proteins and confers protection from metabolic inhibition to cardiomyocytes. *J. Biol. Chem.* 278: 20235-20239, 2003.

### **Discovery and Characterization of a Novel ABC Transporter Gene.**

The Abca13 gene encodes an ATP-binding cassette (ABC) transporter with 12 transmembrane domains that is expressed at relatively high levels in the epididymis and salivary gland. Of the 48 members of the human ABC transporter gene superfamily, 14 are associated with human diseases (including cystic fibrosis, retinal degeneration, and sterol transport deficiencies) and 6 are associated with multidrug resistance (MDR). The orthologous human ABCA13 gene maps to a syntenic region on chromosome

7p12.3, containing a locus (INM7) associated with T-cell tumor invasion and metastasis, which is presently under investigation by NIEHS scientists.

Barros, S.A., Tennant, R.W. and Cannon RE.: Molecular structure and characterization of a novel murine ABC transporter, Abca13. *Gene* 307:191-200, 2003

**Development of a yeast system to determine fidelity of DNA replication.**

NIEHS scientists have established an in vivo system using yeast cells to determine the fidelity of DNA replication by the leading and lagging strand DNA replication machinery and used the system to demonstrate that yeast origins establish a strand bias for replicational mutagenesis induced by two base analogs, one of which (8-oxo-guanine) is a major DNA lesion generated by oxidative stress.

Pavlov, Y.I., Newlon, C.S. and Kunkel, T.A.: Yeast origins establish a strand bias for replicational mutagenesis. *Molec. Cell* 10, 207-213, 2002.

**Mechanism for Control of Antibody Affinity.**

NIEHS scientists obtained evidence that DNA polymerase eta, a Y family enzyme, likely participates in somatic hypermutation of immunoglobulin genes, a process responsible for development of high affinity antibodies.

Pavlov, Y.I., Rogozin, I.B., Galkin, A.P., Aksenova, A.Y., Hanaoka, F., Rada, C. and Kunkel, T.A.: Evidence for participation of DNA polymerase eta in somatic hypermutation of an immunoglobulin  $\kappa$  light chain transgene. *Proc. Natl. Acad. Sci. USA* 99, 9954-9959, 2002.

**A Mathematical Model Indicates that Current Risk Assessment Procedures May Not be Adequate for Environmental Agents that Mimic Natural Hormones.**

NIEHS scientists created mathematical model to examine how environmental agents that bind to nuclear receptor proteins may affect the expression of hormone-regulated genes. The model indicates that a non-monotonic response is a plausible outcome for environmental agents that activate nuclear receptors (e.g., estrogen receptor) in the same manner as the natural hormone (e.g., estrogen). These results imply that such agents can have important consequences on human health even at low, environmental exposure levels

Kohn, M.C. and Melnick, R.L.: Biochemical origins of the non-monotonic receptor-mediated dose-response. *J. Mol. Endocrinol.* 29: 113-123, 2002.

**Genetic Mutations in Mice Offer Clues About Cancer-Causing Pathways Following Chemical Exposure.**

Over 30 million pounds of o-nitrotoluene are produced in the United States each year because of its use in synthesizing a wide variety of industrial products including pesticides, pharmaceuticals, dyes, and rubber chemicals. Researchers at the NIEHS report that a diet containing o-nitrotoluene caused hemangiosarcomas, a cancer originating from blood vessels at multiple sites (skeletal muscle, mesentery, subcutaneous tissues), in mice. The significance of the study is the different genetic mutations found between the hemangiosarcomas caused by exposure to the o-nitrotoluene and similar tumors that occurred spontaneously, without chemical induction. The researchers examined the genetic mutations in three genes for which mutations are thought to be important in the development of human cancers. These findings offer important clues to how cancer may occur in people when exposed to this or other potentially toxic substances.

Hong, H.L., Ton, T.V., Devereux, T.R., Moomaw, C., Clayton, N., Chan, P., Dunnick, J.K. and Sills, R.C.: Chemical-specific alterations in ras, p53, and B-catenin genes in hemangiosarcomas from B6C3F1 mice exposed to o-nitrotoluene or reddelline for 2 years. *Tox. Appl. Pharmacol.*, in press, 2003.

#### **A Biological Model Characterizes Methemoglobinemia in Animals and Humans.**

NIEHS scientists created biologically based mathematical model to characterize the induction of methemoglobinemia due to exposure to nitrite. Elevated levels of methemoglobin can lead to anemia hypoxia, a condition in which there is inadequate supply of oxygen to tissues. The model predicts the rate and extent of oxidation of hemoglobin by nitrite in rats given oral doses of sodium nitrite. The model also predicts the induction and recovery of methemoglobinemia in humans

Kohn, M.C., Melnick, R.L., Ye, F. and Portier, C.J.: Pharmacokinetics of sodium nitrite-induced methemoglobinemia in the rat. *Drug Metab Dispos.* 30: 676-683, 2002.

#### **Direct Comparison of Results From Human Studies of PCB Exposure in Relation to Neurodevelopment Made Possible.**

Everyone in developed countries is exposed before birth to the man-made environmental contaminant, PCB, which is known to adversely effect neurodevelopment in animals. Whether exposure to low levels of PCB is harmful to humans is controversial and affects regulatory decisions, hazardous waste cleanup policy, and dietary recommendations. NIEHS researchers have facilitated risk assessment and data interpretation by making direct comparison of results across studies possible using original data obtained from all investigators in this field, and produced results that weaken the evidence that low-level exposures in humans may have adverse effects.

Daniels, J.L., Longnecker, M.P., Klebanoff, M.A., Gray, K.A., Brock, J.W., Zhou, H., Chen, Z. and Needham, L.L.: Prenatal exposure to low level polychlorinated biphenyls in relation to mental and motor development at 8 months. *Am. J. Epidemiol.*, 157: 485-92, 2003.

Longnecker, M.P., Wolff, M.S., Gladen, B.C., Brock, J.W., Grandjean, P., Jacobson, J.L., Korrick, S.A., Rogan, W.J., Weisglas-Kuperus, N., Hertz-Picciotto, I., Ayotte, P., Stewart, P., Winneke, G., Charles, M.J., Jacobson, S.W., Dewailly, E., Boersma, E.R., Altshul, L.M., Heinzow, B., Pagano, J.J. and Jensen, A.A.: Comparison of Polychlorinated Biphenyl Levels across Studies of Human Neurodevelopment. *Environ. Health Perspect.* 111: 65-70, 2003.

### **Gene Expression Changes Found in Individuals Accidentally Exposed to Dioxin in 1976.**

A long-term collaborative research study between the NIEHS, the National Cancer Institute, the Centers for Disease Control and Prevention and Italian investigators at the University of Milan and the Hospital of Desio examined gene expression in people environmentally exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a result of an industrial accident in Seveso, Italy in 1976. This population-based study evaluated the impact of TCDD exposure on mechanistically based biomarkers of dioxin response in peripheral blood mononuclear cells from approximately 120 men and women. Changes in gene expression were analyzed with respect to serum TCDD levels, genetic polymorphisms, demographic variables such as age and gender, and experimental variables related to laboratory techniques. In this study, expression of the gene encoding the aryl hydrocarbon receptor, a TCDD-activated transcription factor, and cytochrome P4501A1 dependent enzyme activity was negatively associated with TCDD body burden. Furthermore, in vitro inducibility of cytochrome P4501B1 gene expression was found to be associated with variant alleles of the CYP1B1 gene. Together, these findings provide valuable data describing variability in gene expression in TCDD-exposed individuals, highlight the importance of accounting for laboratory measurement variability in molecular epidemiology studies, and identify genetic determinants of human variability in response to dioxin exposure and possible adverse health outcomes.

Landi, M.T., Bertazzi, P.A., Baccarelli, A., Consonni, D., Masten, S.A., Mocarelli, P., Patterson, D.G. Jr., Needham, L.L., Lucier, G., Caporaso, N. and Grassman, J.A.: TCDD-mediated alterations in AHR-dependent pathways in Seveso, 20 years after the accident. *Carcinogenesis*, 24: 673-680, 2003.

Landi, M.T., Baccarelli, A., Bertazzi, P.A., Pesatori, A., Consonni, D., Caporaso, N., Patterson, D.G. Jr., Needham, L.L., Mocarelli, P., Grassman, J.A., Masten, S.A. and Lucier, G.W.: Correspondence re: Toide et al., Aryl Hydrocarbon Hydroxylase represents CYP1B1, and not CYP1A1, in human freshly isolated white cells: trimodal distribution of Japanese population according to induction of CYP1B1 mRNA by environmental dioxins. *Cancer Epidemiol., Biomark. Prev.*, in press.

### **Chromatin structure in *Drosophila* telomeres is regulated by mechanisms that control telomere length.**

Chromatin structure in telomeric regions can be monitored using a transgene inserted into a telomere. Expression of a telomeric white reporter gene increases in response to deletion of the telomere associated sequence (TAS) on the homologue, but only when the reporter is next to a terminal transposon array that includes at least one complete HeT-A element. The level of expression, which is a function of the number of highly expressing spots, increases with the number of HeT-A elements in cis. It should be noted in this regard that telomere length in *Drosophila* is maintained by the targeted transposition of the non-LTR retrotransposons, HeT-A and TART to chromosome ends, and that transcription is the first step in the retrotransposition cycle. Thus, the transcriptional activation of HeT-A seems to counteract the previously described telomeric silencing that spreads from TAS in cis.

Mason, J.M., Konev, A.Y., Golubovsky, M.D. and Biessmann, H.: Cis- and trans-acting influences on telomeric position effect in *Drosophila melanogaster* detected with a subterminal transgene. *Genetics*, 163:917-930, 2003.

#### **Elucidation of the Mechanism of Brain Formation**

It has been believed for long that BMPs play critical roles for formation of neural tissues, but lack of proper in vivo models has prevented the exploration of actual mechanisms. NIEHS scientists have established a knock-out mouse model of one of the BMP receptors, BMPRIa, specifically in the most dorsal section of the telencephalon. This unique model has provided evidence that BMP is required for formation of the most dorsal structure of telencephalon, choroid plexus.

Hebert, J.M., Mishina, Y. and McConnell, S.K.: BMP signaling required locally to pattern the dorsal telencephalic midline. *Neuron* 35:1029-1041, 2002.

#### **Damage To DNA Can Lead to Cancer and Heritable Birth Defects Unless Repaired.**

Over the past several decades, several ways have been discovered by which such damage can be repaired. However, no fundamentally new mechanism has surfaced for many years. Now, a process called "replication repair" has been described by NIEHS scientists. The DNA damage is circumvented rather than removed, by a process in which a growing DNA strand jumps to an alternative template--the DNA strand already copied from the other parental DNA strand.

Kadyrov, F.A. and Drake, J.W.: Properties of bacteriophage T4 proteins deficient in replication repair. *J. Biol. Chem.*, 278:25247 - 25255, 2003.

#### **Large New Prospective Cohort, The National Children's Study, Being Planned To Evaluate Environmental Effects On The Health Of Children.**

In response to the Children's Health Act of 2000, investigators at NICHD, NIEHS, EPA, and CDC are planning a large new cohort study of the determinants of child health. The planning process includes a careful evaluation of priorities in child health and the design

of a national study that will allow huge advances in studying the determinants of child health.

The National Children's Study Interagency Coordinating Committee. The National Children's Study of Environmental Effects on Child Health and Development. Environ. Health Perspect. 111:640-6, 2003.

**Inactivation of a NADH Kinase Gene Involved in the Natural Defense Against Oxidative Stress Causes Mutations in Mitochondrial DNA.**

Using a new methodology to assay the genetic stability of mitochondrial genomes readily, NIEHS scientists have identified a mitochondrial NADH kinase (Pos5) in *Saccharomyces cerevisiae* that functions to protect mitochondria against oxidative stress. The Pos5 is used as part of our natural anti-oxidant defenses and mutation of this gene can cause an increase in mutations in the mitochondrial genome by over 50-fold.

Strand, M.K., Stuart, G., Longley, M.J., Graziewicz, M.A., Dominick, O.C. and Copeland, W.C.: POS5 gene of *Saccharomyces cerevisiae* encodes a mitochondrial NADH kinase required for stability of mitochondrial DNA. Eukaryotic Cell, 4: in press, 2003.

**Computational Evidence For a Protective Mechanism Against Common Cause of Mutations in Human Genome.**

Human DNA is constantly subjected to environmental forces that result in mutations and some of these mutations can eventually lead to serious diseases such as cancer. By analyzing several million DNA patterns surrounding human genetic variations, NIEHS scientists have uncovered "fingerprint" sequences that reveal the impact of important mutation mechanisms. Existing theories of mutagenesis have been validated using this approach. Genome features called "CpG Islands" are apparently protected from mutation. This analysis provides new insights into the forces that govern variation in the human genome, represents a substantial advance in our capability to perform high-throughput computational analysis of the human genome, and has generated numerous valuable leads for ongoing studies of human disease.

Tomso, D.J. and Bell, D.A.: Sequence context at human single nucleotide polymorphisms: overrepresentation of CpG dinucleotide at polymorphic sites and suppression of variation in CpG islands. J. Mol. Biol. 327:303-308, 2003.

**Metabolic Pathway Differences For Nitrotoluene Isomers Lead to Different Carcinogenic Outcomes.**

O-Nitrotoluene and p-Nitrotoluene are structurally related chemicals, differing only in the placement of the nitro group on the aromatic ring. NIEHS toxicologists found that o-nitrotoluene caused a broad spectrum of cancer in rodent models including colon carcinomas, hemangiosarcomas, skin tumors, and mammary tumors. In contrast, p-

nitrotoluene did not cause these cancers. The metabolic pathways for o-nitrotoluene and p-nitrotoluene differ, and only the ortho isomer is capable of forming a carcinogenic metabolite. These studies show that understanding differences in metabolism of environmental chemicals can be used to predict carcinogenic outcome.

Dunnick, J.K., Burka, L.T., Mahler, J. and Sills, R.: Carcinogenic potential of o-nitrotoluene and p-nitrotoluene. *Toxicology* 183, 221-234, 2003.

#### **Rat Central Serotonin 5-HT<sub>3</sub> Receptors: Functional and Molecular Characterization in Rat Hippocampus, and Regulation by Casein Kinase II.**

Using electrophysiological and molecular techniques, NIEHS scientists have found evidence, at least in some cells, for the co-expression and co-assembly of the 5-HT<sub>3A-short</sub> and  $\alpha 4$  n-acetylcholine receptor subunits, the first demonstration of co-assembly of subunits from diverse ligand-gated ion channels in vivo. However, no functional or molecular evidence for the heteromeric co-assembly of the 5-HT<sub>3A</sub> and 5-HT<sub>3B</sub> subunits could be found in hippocampal interneurons. In addition, they found no evidence to suggest that either the 5-HT<sub>3B</sub> or the 5-HT<sub>3A-long</sub> subunits are expressed in these neurons. In addition in NG108-15 cells, a model cell line, the 5-HT<sub>3</sub> receptors are directly regulated by the enzyme, casein kinase II, the first such demonstration to date.

Sudweeks, S.N., van Hooft, J.A. and Yakel, J.L.: Serotonin 5-HT<sub>3</sub> Receptors in Rat CA1 Hippocampal Interneurons: Functional and Molecular Characterization. *J. Physiology* 544:715-726, 2002.

Jones, S. and Yakel, J.L.: Casein kinase II (protein kinase CK2) regulates serotonin 5-HT<sub>3</sub> receptor channel function in NG108-15 cells. *Neuroscience* 119: 629-634, 2003.

#### **Diethylstilbestrol Exposure Can Lead to Leiomyomas.**

NIEHS scientists have found that prenatal exposure to diethylstilbestrol (DES) can lead to increased incidence of smooth muscle uterine leiomyomas in mice. These DES-induced leiomyomas have typical histomorphologic and some immunohistochemical characteristics of spontaneously occurring smooth muscle tumors.

Newbold, R.R., Moore, A.B. and Dixon, D.: Characterization of uterine leiomyomas in CD-1 mice following developmental exposure to diethylstilbestrol. *Tox. Pathol.* 30:611-616, 2002.

#### **Determination of the Solution Structure of Ribonuclease H**

NIEHS scientists determined the first solution structure of the Ribonuclease H domain of HIV reverse transcriptase. This enzyme is essential for replication of the human immunodeficiency virus, and contains two catalytic sites – a polymerase site and a second site with RNase H activity. Although there has been extensive drug development targeting the polymerase site, there are currently no clinical drugs that target the RNase H

site. It is anticipated that the availability of the solution structure will contribute toward the development of such drugs.

Pari, K., Mueller, G.A., DeRose, E.F., Kirby, T.W. and London, R.E.: Solution structure of the RNase H domain of the HIV-1 reverse transcriptase in the presence of magnesium. *Biochemistry* 42:639-650, 2003.

#### **Development of a Specific Boronic Inhibitor of $\gamma$ -Glutamyl Transpeptidase**

Boron is an essential trace element but at higher levels exhibits significant toxicity, particularly to male reproductive function. Based on proposed ternary borate complexes with the enzyme  $\gamma$ -glutamyl transpeptidase, NIEHS scientists have developed a specific boronic acid inhibitor of this enzyme. During the past year, they obtained the first evidence for the covalent binding of borate to an enzyme, in this case trypsin, as a ternary complex. The demonstration of ternary covalent complexes involving enzymes, alcohols and borate provides a potential basis for the physiological activity as well as the toxicity of borate.

London, R.E. and Gabel, S.A.: Formation of a trypsin-borate-4-aminobutanol ternary complex. *Biochemistry* 41:5963-5967, 2002.

Transue, T.R., Krahn, J.M., Gabel, S.A., DeRose, E.F. and London, R.E.: Crystal structures of ternary and quaternary complexes formed from trypsin, borate, and alcohols, in preparation.

#### **Dynamic Behavior of the Glucocorticoid Receptor Probed.**

NIEHS scientists studied the mobility of the glucocorticoid receptor in the nuclei of living cells using fluorescence recovery after photobleaching (FRAP). They found that ligand binding decreases mobility of the receptor and that the extent of this decrease can vary in an affinity-dependent manner for different ligands. Furthermore, they found that DNA-binding and ligand-binding domains both play a role in this decreased mobility. These results suggest that ligand binding to glucocorticoid receptor may cause a conformational change that targets the receptor to relatively immobile nuclear domains, and provides new insight on the behavior of this receptor system in the nucleus.

Schaaf, M.J.M. and Cidlowski, J.A.: Molecular determinants of glucocorticoid receptor mobility in living cells: the importance of ligand affinity. *Mol. Cell. Biol.* 23:1922-1934, 2003.

#### **Development of Potent Opioid Mimetic Agonists and Antagonists.**

NIEHS scientists designed a new class of opioid mimetic compounds, substances that interact within the brain similar to the action of morphine, that are stable when injected peripherally in mice. Essentially, the material consists of two unusual amino acids tethered together by a simple carbon chain. The simplicity of this new material underscores its effectiveness for potential application in clinical treatment or veterinary

applications. Based on those results, another new group of similar synthetic compounds exerted oral bioavailability; that activity was approximately half of that found with morphine. This indicates that this substance was absorbed through the gut and transported in the blood to the brain where it crossed the blood-brain barrier to produce analgesia. Further, subtle modification of another family of antagonists, which had been transformed into an agonist, reverted back to an antagonist; however, in this conversion the compounds became even more potent antagonists by factors of 5 to 10. These results indicate that small, discrete changes in an opioid substance can exert profound effects on its activity. Several of these compounds are under review for patent protection in both the U.S. and in Japan.

- Okada, Y., Tsuda, Y., Yokoi, T., Sasaki, Y., Ambo, A., Nagata, M., Yunden, J., Bryant, S.D. and Lazarus, L.H.: Unique high affinity synthetic  $\delta$ -opioid receptor agonists with central- and systemic-mediated analgesia. *J. Med. Chem.*, on-line 16 June, 2003.
- Okada, Y., Jinsmaa, Y., Miyazuki, A., Fukita, Y., Fujisawa, Y., Shiotani, K., Li, T., Tsuda, Y., Yokoi, T., Ambo, A., Sasaki, Y., Bryant, S.D. and Lazarus, L.H.: Oral availability of a new class of analgesics. *Nature*, submitted, 2003.
- Balboni, G., Salvadori, S., Guerrini, R., Negri, L., Giannini, E., Bryant, S.D., Jinsmaa, Y. and Lazarus, L.H.: Synthesis and opioid activity of N,N-dimethyl-Dmt-Tic analogues: acquisition of potent  $\delta$  antagonism. *Bioorg. Med. Chem.* submitted, 2003.

#### **Molecular Model of the $\delta$ -opioid Receptor and Docking of Opioid Ligands.**

A computer-generated model of the  $\delta$ -opioid receptor based mutations introduced into the X-ray diffraction structure of bovine rhodopsin revealed new residues buried within the receptor that may be responsible for the differential action of agonists and antagonists.

- Bryant, S.D., Okada, Y., Tsuda, Y., Fujita, Y., Yokoi, T., Yunden, J. and Lazarus, L.H.: Molecular modeling of structurally related bioactive  $\delta$ -opioidmimetics: parameters defining  $\delta$ - and  $\mu$ -receptor selectivity. In, Benedetti E, Rocchi R (eds.) *Peptides 2002*, in press 2003.
- Bryant, S.D., Salvadori, S., Guerrini, R., Balboni, G., Yunden, J. and Lazarus, L.H.: Computational docking and opioidmimetics: investigation of  $\delta$ -opioid agonist and antagonist receptor interactions. In, Benedetti E, Rocchi R (eds.) *Peptides 2002*, in press 2003.
- Bryant, S.D., Yunden, J., Salvadori, S., Okada, Y. and Lazarus, L.H.: Dmt and opioid peptides: a potent alliance. *Biopolymers/Peptide Science*, 71, 86-102, 2003.

#### **Dogma of Chemically Induced Somatic Mutagenesis: Germ Cell Mutagenesis Refuted With Implications For Risk of Acrylamides in Foods.**

NIEHS scientists have shown that NHMA induced high levels of genetic damage in mouse germ cells with no induction of mutations in somatic cells of these same mice.

These results refuted the long held dogma that all chemicals which cause heritable genetic damage in germ cells, that can lead to birth defects, infertility and predisposition to cancer, also cause somatic cell mutations, that can lead to cancer. These results indicates the need for the NTP to evaluate chemicals for their germ cell mutagenicity independent of their somatic cell mutagenicity or lack thereof and also suggest this acrylamide congener bioaccumulates such that total accumulated dose is critical for its adverse effect; this may have important implications for assessing the human health risk of chronic exposures to acrylamides in foods.

Witt, K.L., Hughes, L.A., Burka, L.T., Mcfee, A.F., Mathews, J.M., Black, S.L. and Bishop, J.B.: Mouse bone marrow micronucleus test results do not predict the germ cell mutagenicity of n-hydroxymethylacrylamide in the mouse dominant lethal assay, *Environ. Molec. Mutagen.* 41:111-120, 2003.

### **Correcting the Draft Human Genome**

NIEHS scientists have shown that some errors in the draft human genome sequence are the results of both mis-assembly and loss of specific DNA sequences during cloning in *E. coli*. Their results suggest that transformation-associated recombination cloning in yeast might be a valuable method that could be widely used during the final stages of the Human Genome Project to isolate missing DNA segments.

Kouprina, N., Leem, S.H., Solomon, G., Ly, A., Koriabine, M., Otstot, J., Pak, E., Dutra, A., Zhao, S., Barrett, J.C. and Larionov, V.: Segments missing from the draft human genome sequence can be isolated by transformation-associated recombination cloning in yeast. *EMBO Rep.* 4:257-262, 2003.

## DIR AWARDS AND HONORS

- Dr. David Armstrong (Laboratory of Signal Transduction) was named a Guest Professor in the Department of Molecular Neurobiology at the University of Salzburg and will give a course on cell signaling in the nervous system.
- Dr. Jan Drake (Chief, Laboratory of Molecular Genetics) was elected President of the International Genetics Federation for 2003-2008.
- Dr. David Dunson (Biostatistics Branch) won the "Best Paper Award" from the American Academy of Fertility Care Professionals.
- Dr. E. Mitch Eddy (Laboratory of Reproductive and Developmental Toxicology) was elected to the Board of Directors, American Society for the Study of Reproduction (2002-2005) and the Executive Council, American Society of Andrology (2003-2006), appointed Associate Editor of Biology of Reproduction, and invited to be an Australian Research Centre Scholar, Australian Centre of Excellence in Biotechnology and Development, Monash Institute of Reproduction and Development, Monash University in 2004.
- Dr. Ronald Mason (Laboratory of Pharmacology and Chemistry) gave the Lawrence H. Piette Memorial Lecture, at the 44th Rocky Mountain Conference on Analytical Chemistry - Denver, CO entitled "In Vivo Lipid-derived Free Radical Formation by NADPH Oxidase in Acute Lung Injury Induced by Lipopolysaccharide - a Model for ARDS."
- Dr. Ron Melnick (National Toxicology Program) has been named to Who's Who in America.
- Dr. Fred Miller gave the Kovacs Lecture at the Royal Society of Medicine, London, UK in March 2003 entitled "New Developments in Pathogenesis and Therapy of the Idiopathic Inflammatory Myopathies".
- Dr. Christopher Portier (Chief, Laboratory of Computational Biology and Risk Analysis) was selected to give the Keynote Lecture, Conference on Mechanistic Modeling of Carcinogenesis, Japanese Biometrics Society and Radiation Effects Research Foundation, Kyoto, Japan, March 2003.
- Dr. Lisa Rider (Office of Clinical Research) gave the Schlager Family Visiting Professor Lectureship in Juvenile Dermatomyositis at Children's Hospital, Boston, MA in April, 2003 entitled "Juvenile Idiopathic Inflammatory Myopathies: Lessons from the Children."
- Dr. Steven Shears (Laboratory of Signal Transduction) was named keynote speaker at the second Japan/Korea conference on cellular signaling, held at Kyushu University, Fukuoka, Japan in June 2003 and appointed to the editorial board of the reviews journal *Essays in Biochemistry*.
- Dr. Raymond Tennant (Director, National Center for Toxicogenomics) served as the Co-Chair of the Inaugural Gordon Conference on Toxicogenomics held at Bates College, Lewiston, Maine in June 2003 and was the Keynote Speaker at the NordTox Meeting in Bornholm, Denmark in June 2003.
- Dr. Samuel Wilson (Deputy Director and Laboratory of Structural Biology) was the Keynote Speaker at the American Chemistry Council-LRI First Annual Science Meeting and at the Gordon Research Conference on Toxicogenomics; served as a member of the Editorial Board for the Annual Reviews of Medicine and as an

Associate Editor for DNA Repair; and served on the Program Committee for the 9<sup>th</sup> International Conference on Environmental Mutagens, San Francisco, CA; as the Co-Chair of the Biannual US-EU DNA Repair Meeting; as Director of the Radiation Effects Research Foundation (A Cooperative Japan-United States Research Organization managed in the US by the NAS); and as Co-chair of “Advances in Toxicogenomics: NIEHS National Center for Toxicogenomics,” a Symposium at the Society of Toxicology Annual Meeting in March 2003.

Dr. Jerrel Yakel (Laboratory of Signal Transduction) has been named to the Editorial Board of the Journal of Molecular Neuroscience.

Dr. Darryl Zeldin (Laboratory of Pulmonary Pathobiology) was named to the Editorial Board of the journal Prostaglandins and Other Lipid Mediators

## **NATIONAL TOXICOLOGY PROGRAM (NTP) UPDATE**

### **Dr. William Stokes Named Chief Veterinary Officer for PHS**

NIEHS' Capt. William (Bill) Stokes has been named veterinarian chief professional officer for the Public Health Service Commissioned Corps. The appointment, by U.S. Surgeon General Richard H. Carmona, went into effect on May 1. As the chief veterinary officer, Dr. Stokes will coordinate veterinary professional affairs and oversee recruitment, retention, career development and readiness of the more than 100 PHS veterinary officers, stationed throughout the NIH, the Centers for Disease Control and Prevention, the FDA, and other federal agencies. Dr. Stokes will continue to direct the NTP's Interagency Center for the Evaluation of Alternative Toxicological Methods, which has led the federal government's reform of animal testing by introducing the use of scientifically acceptable non-animal testing to replace tests that expose animals to harsh chemicals.

### **NTP Launches New Technical Report Series**

The NTP launched a new technical report series to be called Genetically Modified Models, or GMM series at the May 22 meeting of the NTP Board of Scientific Counselors Technical Reports Review Subcommittee, a standing subcommittee of the NTP Board of Scientific Counselors. At that meeting, the Subcommittee peer reviewed the draft findings and conclusions from the first two NTP technical reports in this series, aspartame (GMM-1) and acesulfame potassium (GMM-2). This new series will contain the results from NTP toxicology and carcinogenicity studies conducted in genetically modified models, such as transgenic mice that have had a key gene related to cancer or other diseases added, "knocked out" or slightly changed.

The actions from this meeting, including the subcommittee's recommendations on the findings and conclusions from carcinogenicity studies on aspartame and acesulfame potassium in p53 haploinsufficient mice as well as four other NTP studies conducted in traditional rodent models - 2-methylimidazole, propylene glycol mono-*t*-butyl ether, stoddard solvent IIC and triethanolamine - are available on the web (<http://ntp-server.niehs.nih.gov/Meetings/2003/May2003Actions.html>). As the final reports from this new series are published, they will be available along with reports from the NTP Technical Report and NTP Toxicity Report series in hardcopy and electronic format from Environmental Health Perspectives (<http://ehp.niehs.nih.gov>). The electronic files are available free-of-charge.

## **NTP Board of Scientific Counselors**

The annual meeting of the NTP Board of Scientific Counselors is scheduled for September 10-11, 2003 at the NIEHS. The NTP Board of Scientific Counselors (“the Board”) is composed of scientists from the public and private sector and provides primary scientific oversight to the NTP. Primary agenda topics include: 1) a vision for the NTP that includes its concept and projection of new areas into which toxicology will develop in the next 5-10 years; 2) a presentation on the development of new, publicly accessible, electronic databases for NTP studies; 3) a demonstration of an interactive, web-based, 2-D-imaging system to evaluate the pathological outcomes of NTP studies; and 4) updates on the NTP testing program including the design of studies on radio-frequency radiation from cellular phone devices, collaborations with the National Institute of Occupational Safety and Health, studies on medicinal herbs and dietary supplements and the recommendations of the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) for substances nominated to the NTP for study. There will also be updates on the NTP Board of Scientific Counselors Technical Reports Peer Review Meeting held on May 22, 2003, the status of the 11<sup>th</sup> Edition of the Report on Carcinogens and the NTP Center for the Evaluation of Risks to Human Reproduction. Time is allotted during the meeting for the public to present comments to the Board and NTP staff on agenda topics.

### **NTP Board of Scientific Counselors Report on Carcinogens Subcommittee**

A meeting of the NTP Board of Scientific Counselors Report on Carcinogens Subcommittee, a standing subcommittee of the NTP Board of Scientific Counselors is scheduled for October 14-15, 2003 at the Marriott at Metro Center in Washington, DC. Scheduled for peer review are the second set of nominations to the 11<sup>th</sup> Edition of the Report on Carcinogens: diazoaminobenzene, hepatitis B virus, hepatitis C virus, human papillomaviruses (genital-mucosal types), X-radiation and gamma-radiation, neutrons, and lead and lead compounds. Background documents for these nominations, public comments received on them and details about the meeting are posted on the NTP web site at <http://ntp-server.niehs.nih.gov/Meetings/2003/2003OctRoC11Mtg.html>

### **Digitized Atlas of Rodent Kidney Lesions**

The Laboratory of Experimental Pathology, Environmental Toxicology Program, announces the availability of a digitized atlas of rodent (rat and mouse) kidney lesions and lower urinary tract lesions. The purpose of this atlas is to familiarize pathologists and others with the spontaneous and chemically induced lesions seen in the kidneys of laboratory rodents. It contains a list of references on lesions of the rodent kidney and lower urinary tract. This atlas is available on the NTP web site at [http://ntp-server.niehs.nih.gov/Main\\_Pages/RodentModLesions.html](http://ntp-server.niehs.nih.gov/Main_Pages/RodentModLesions.html)

The web site also links to two additional atlases released previously: *A Digitized Atlas of Mouse Liver Lesions* and *Lesions of Genetically Altered Mice*.

## NTP Study Nominations

The NTP continuously solicits and accepts nominations for toxicological studies to be undertaken by the program. The nominations are subjected to several levels of review before selections for testing are made and toxicological studies are designed and implemented. As part of this review process, the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) met on June 10, 2003, to review 14 new nominations and make study recommendations. Internet links to electronic versions of supporting documents for each nomination and further information on the NTP and the NTP Chemical Nomination and Selection Process can be accessed through the NTP web site. The NTP is currently soliciting public comments on the nominations and study recommendations (Federal Register July 16, 2003: Vol. 68, No. 136, pages 42068 – 42071). The public is also invited to provide oral comments at the September 10-11, 2003 meeting of the NTP Board of Scientific Counselors.

### Substances recommended for testing by the ICCEC

- Acrylamide [79-06-1] and Glycidamide [5694-00-8]: recommended studies - toxicological characterization, toxicokinetics, mechanistic (hemoglobin adducts), carcinogenicity and bioavailability from food and drinking water.
- Antimony trisulfide [1345-04-6]: recommended studies: chronic toxicity/carcinogenicity.
- Cadmium telluride [1306-25-8]: recommended studies - toxicological characterization and chemical disposition (oral and inhalation routes).
- Cedarwood oil, Virginia [8000-27-9]: recommended studies - toxicological characterization and developmental toxicity.
- Chondroitin sulfate [9007-28-7]: recommended studies - chronic toxicity/carcinogenicity and carcinogenicity of chondroitin sulfate and glucosamine combined.
- Dimethylethanolamine [108-01-0]: recommended study – metabolism.
- Drugs positive for QT Interval Prolongation/ Induction of *Torsade* Proarrhythmia [No CAS No.]: recommended studies - initiate a study program to develop *in vitro* and *in vivo* test systems for assessing QT interval prolongation.
- Glucosamine [3416-24-8]: recommended studies - chronic toxicity/carcinogenicity and carcinogenicity of chondroitin sulfate and glucosamine combined.
- Nanoscale materials [No CAS No.]: recommended studies – size- and composition-dependent biological disposition of nanocrystalline fluorescent semiconductor materials, toxicological characterization of high-aspect-ratio carbon nanomaterials,

role of particle core and surface composition in the immunotoxicity of the above listed materials, and phototoxicity of representative metal oxide nanoparticles.

- *trans*-Resveratrol [501-36-0] recommended studies - toxicological characterization, carcinogenicity and reproductive toxicity.
- Tetrabromobisphenol A [79-94-7]: recommended studies - toxicological characterization, neurodevelopmental toxicity, and carcinogenicity.
- Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) [21850-44-2]: recommended studies - toxicological characterization, *in vivo* genotoxicity, metabolism and carcinogenicity.
- Tungsten [7440-33-7]: recommended studies - toxicological characterization and carcinogenicity; studies should focus on a representative soluble tungsten compound.

**Substance for which the ICCEC recommends no study at this time**

- 4-Phenylcyclohexene [4994-16-5]: low suspicion of hazard based on available human exposure and toxicity information.

## **NTP Interagency Center for the Evaluation of Alternative Toxicological Methods**

The following report, *ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays* (NIH No. 03-4503) is available on the NICEATM/ICCVAM web site (<http://iccvam.niehs.nih.gov>) or by contacting NICEATM ([niceatm@niehs.nih.gov](mailto:niceatm@niehs.nih.gov)). It contains recommendations by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) on minimum procedural standards and reference chemicals for standardization and validation of *in vitro* estrogen and androgen receptor binding and transcriptional activation assays. The Environmental Protection Agency asked ICCVAM to evaluate the validation status of these assays proposed as possible components of the EPA Endocrine Disruptor Screening Program (EDSP) Tier 1 screening battery. ICCVAM agreed to this evaluation based on their potential interagency applicability and public health significance.

The ICCVAM Dermal Corrosivity and Irritation Working Group has proposed Minimum Performance Standards (MPS) for three types of *in vitro* methods used for assessing the dermal corrosivity hazard potential of chemicals. The ICCVAM developed the proposed MPS to communicate criteria that can be used to determine if similar test methods have comparable accuracy and reliability. After the public comment period (Federal Register July 1, 2003: Vol. 68, No. 126, pages 39104-5) ends and the report is finalized, ICCVAM MPS will be published as an addendum to previously published ICCVAM reports on these test methods and forwarded to federal agencies for their consideration. Copies of the MPS will be made available electronically on the ICCVAM/NICEATM web site or in hardcopy by contacting NICEATM.

ICCVAM and NICEATM are collaborating with the European Centre for the Validation of Alternative Methods (ECVAM) to conduct a validation study on *in vitro* test methods for assessing dermal irritation. NICEATM is soliciting chemical and protocol information/test data on commercially available chemicals used for dermal or ocular irritancy in rabbits and/or for dermal irritancy in humans using standardized testing methods (Federal Register July 16, 2003: Vol. 68, No. 136, pp. 42067-8) . ICCVAM and its Dermal Corrosivity and Irritation Working Group will review the data and identify chemicals that might be appropriate for use in the upcoming validation study. The resulting list of chemicals tested for skin irritancy in rabbits and/or humans and supporting data will also be provided to ECVAM its consideration.

NICEATM and the ECVAM are conducting a collaborative validation study to evaluate two *in vitro* basal cytotoxicity assays proposed for predicting starting doses for *in vivo* acute oral toxicity assays and lethal concentrations in humans. Three laboratories are participating in the evaluation of the neutral red uptake assays using both a mouse cell line (*i.e.*, BALB/c 3T3 fibroblasts) and a primary human cell line (*i.e.*, normal human epithelial keratinocytes). The cytotoxicity results for 72 coded chemicals, representing a wide range of toxicity, will be used to predict starting doses for the *in vivo* acute oral toxicity assays. Phase I testing was completed in May 2003. All labs have Phase II protocols in place and testing of nine coded chemicals began on June 2. Following

completion of Phase II, final optimized protocols will be prepared and used for Phase III, which will involve testing 60 coded chemicals.